Patent Abstracts of Japan

PUBLICATION NUMBER : 07000181
PUBLICATION DATE : 06-01-95

APPLICATION DATE : 18-06-92
APPLICATION NUMBER : 04182799

APPLICANT : TAGUCHI FUMIAKI;

INVENTOR : OOKUBO HANAKO;

INT.CL. : C12N 1/38 //(C12N 1/38 , C12R 1:44)

TITLE : MEDIUM FOR SELECTIVE CULTURE OF STAPHYLOCOCCUS HAVING MULTIPLE DRUG RESISTANCE

ABSTRACT : PURPOSE: To obtain a culture medium enabling the selective proliferation and detection of Staphylococcus exhibiting multiple drug resistance.

CONSTITUTION: This medium for the culture of Staphylococcus having multiple drug resistance is produced by incorporating 1ml of a medium for the selective culture of Staphylococcus with ≥1.6μg of an oxacillin-type antibiotic substance and ≥6.25μg of a ceftizoxime-type antibiotic substance as antibiotics for determining the multiple drug resistance.

COPYRIGHT: (C)1995,JPO

PATENT ABSTRACTS OF JAPAN

(11)Publication number : **07-000181**
(43)Date of publication of application : **06.01.1995**

(51)Int.CI. C12N 1/38
//(C12N 1/38
C12R 1:44)

(21)Application number : **04-182799**
(22)Date of filing : **18.06.1992**

(71)Applicant : **KYOKUTO SEIYAKU KOGYO KK**
TAGUCHI FUMIAKI
(72)Inventor : **TAGUCHI FUMIAKI**
MIYAO HITOSHI
OOKUBO HANAKO

(54) MEDIUM FOR SELECTIVE CULTURE OF STAPHYLOCOCCUS HAVING MULTIPLE DRUG RESISTANCE

(57)Abstract:
PURPOSE: To obtain a culture medium enabling the selective proliferation and detection of Staphylococcus exhibiting multiple drug resistance.
CONSTITUTION: This medium for the culture of Staphylococcus having multiple drug resistance is produced by incorporating 1ml of a medium for the selective culture of Staphylococcus with ≥1.6μg of an oxacillin-type antibiotic substance and ≥6.25μg of a ceftizoxime-type antibiotic substance as antibiotics for determining the multiple drug resistance.

LEGAL STATUS

[Date of request for examination]	09.03.1999
[Date of sending the examiner's decision of rejection]	
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]	
[Date of final disposal for application]	
[Patent number]	3298932
[Date of registration]	19.04.2002
[Number of appeal against examiner's decision of rejection]	
[Date of requesting appeal against examiner's decision of rejection]	
[Date of extinction of right]	

Copyright (C); 1998,2003 Japan Patent Office

* NOTICES *

JPO and NCIPI are not responsible for any
damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The culture medium for multiple drug resistance Staphylococcus culture characterized by including more than 1.6microg and a ceftizoxime system antibiotic per 1ml of culture media, and for an oxacillin system antibiotic more than 6.25microg as an antibiotic for judging multiple drug resistance to the culture medium for cultivating Staphylococcus alternatively.
[Claim 2] Furthermore, the culture medium according to claim 1 which contains per 1ml of culture media, and a gentamycin system antibiotic more than 6.25microg.
[Claim 3] The culture medium for cultivating Staphylococcus alternatively to 1000ml of purified water Casamino acids 14.8-18.2g, the heart extractives 2.7-3.3g, Water soluble chlorophyll derivatives 1.45-1.65g, grape sugars 5-15g, L-tryptophans 0.045-0.055g, The L-cystines 0.045-0.055g, CaCl 20.166-0.202g, MgCl2 0.188-0.23g, NaCl 6.8-8.3g, A culture medium given in claim 1 or the 2nd term which is the thing which comes to add pyruvic-acid sodium 0-15g, 1 - 10% (weight/volume) of yolk liquid, Agars 13.5-16.5g, Mannites 5-20g, and 10-30mg of coloring matter.

[Translation done.]

* NOTICES *

JPO and NCIPI are not responsible for any
damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the culture medium for cultivating alternatively the bacillus in which multiple drug resistance is shown among staphylococci other than Staphylococcus in which multiple drug resistance is shown, i.e., methicillin resistant Staphylococcus aureus, (it is hereafter written as MRSA), and Staphylococcus aureus, for example, coagulase negative Staphylococcus etc., (it is hereafter written as CNS). In order to acquire the therapy guide of the staphylococcia, and information required for the grasp and improvement of a hospital milieu-interne contamination situation for hospital infection prevention in more detail, it is related with the useful above-mentioned culture medium.
[0002]
[Description of the Prior Art] As a bacteria culture medium currently used conventionally, there are SUTAFIRO cox culture-medium No.110, mannite salt ******, a salt egg agar basal medium, etc., and these culture media are applicable to detection of Staphylococcus in specimens, such as patient blood and urine, food, or a hospital environment as a culture medium for separating Staphylococcus alternatively.
[0003] However, although these culture media can proliferate Staphylococcus alternatively, it cannot judge whether it is drug tolerance Staphylococcus. For this reason, in order to have got to know the resistance over drugs, it was required to extract the cluster of Staphylococcus which grew on these Staphylococcus selective media, and to perform a drugs sensitivity test separately about each of clusters (488 J.Clin.Microbiol.18:1084- 1091, 1983, and J.Clin.Microbiol.19:482- 1984). Thus, by the conventional approach, in order to conduct two steps of inspection, inspection days will become long, and an inspection fee will also become high.
[0004] The above-mentioned Staphylococcus selective medium is a culture medium for carrying out isolation culture of Staphylococcus alternatively, and also has the problem that number of microorganism of Staphylococcus which holds the property of the drug tolerance in a specimen cannot be known further again.
[0005] then, Staphylococcus which a culture medium is made to contain antibiotics, such as methicillin (an infectious disease study magazine, 64 volumes, No. 5, the 549 to 556th pages), and ceftizoxime (medicine inspection, 41 volumes, No. 3, the 540th page), independently, and shows resistance to an antibiotic -- although the approach of proliferating alternatively was tried, the thing usable as a culture medium which proliferates multiple drug resistance Staphylococcus alternatively is not yet found out.
[0006] Then, this invention aims at offering the culture medium which can proliferate Staphylococcus which holds a multiple drug resistance property efficiently and alternatively.
[0007]
[Means for Solving the Problem] this invention persons use an oxacillin system antibiotic as index drugs of methicillin resistant bacteria, as a result of repeating examination wholeheartedly about the culture medium for carrying out isolation culture of Staphylococcus in which multiple drug resistance is shown alternatively. This, They are the drugs which can guide methicillin resistance in Staphylococcus of

methicillin susceptibility seemingly. And unlike the drugs of other cephem systems, the inactivated enzyme was hard to be decomposed, and when used for stability combining the ceftizoxime system antibiotic which can exist in a culture medium, it found out that the isolation culture of Staphylococcus in which multiple drug resistance is shown could be carried out alternatively.

[0008] That is, the culture medium of this invention is characterized by including more than 1.6microg and a ceftizoxime system antibiotic per 1ml of culture media, and for an oxacillin system antibiotic more than 6.25microg as an antibiotic for judging multiple drug resistance to the culture medium for cultivating Staphylococcus alternatively.

[0009] Generally, "multiple drug resistance" made beta-lactam system antibiotics, such as methicillin, the start, and has meant that resistance is shown to two or more drugs of all the antibiotics (for example, an aminoglycoside system, a macrolide system, tetracyclines, etc.) used widely now.

[0010] The culture medium of this invention is characterized by containing combining an oxacillin system antibiotic and a ceftizoxime system antibiotic in a culture medium, in order to carry out isolation culture of multiple drug resistance Staphylococcus alternatively. It is indispensable that both contain and effectiveness of this invention cannot be demonstrated only by either being included. As an oxacillin system antibiotic, the methylphenyl isoxazolyl derivative of 6-aminopenicillanic acid etc. is included. Moreover, as a ceftizoxime system antibiotic, it isR [6] and (R[7])-7-[(Z)-2-(2-amino-4- thiazolyl)-2-methoxy imino acetamido]-8. - Oxo-- 5 - Thia -1 - Azabicyclo [4.2.0] oct-2-en -2 - Carboxylic acid, 7-[(Z)-2-(2-amino-4- thiazolyl)-2-methoxy imino acetamido]-3 - Cephem -4 - A carboxylic acid etc. is included. An oxacillin system antibiotic is contained in a culture medium in the amount more than 1.6micro[per 1ml of culture media] g. Since growth of a drugs sensitive strain will be allowed if fewer than this, effectiveness of this invention cannot be demonstrated. Although especially the upper limit of a content is unnecessary, since growth control of a drug-resistant strain may be caused when many [too], below 6.2microg is desirable. The optimum range of the amount used is 1.6-4.0microg per 1ml of culture media. Moreover, a ceftizoxime system antibiotic is contained in a culture medium in the amount more than 6.25microper 1ml of culture media g. Since growth of a drugs sensitive strain will be allowed if fewer than this, effectiveness of this invention cannot be demonstrated. Although especially the upper limit of a content is unnecessary, since growth control of a drug-resistant strain may be caused when many [too], below 25microg is desirable. The optimum range of the amount used is 6.25-12.5microg per 1ml of culture media.

[0011] As for the culture medium of this invention, it is desirable that a gentamycin system antibiotic is included further more than 6.25microg in addition to two sorts of above-mentioned antibiotics again. It is because clinical meaning is large to distinguish whether it is gentamycin resistance also especially in a drug-resistant strain, since it says that many of MRSA stocks with which this is gentamycin resistance and many of MRSA stocks of the inpatient origin are separated for it from health care professionals are gentamycin susceptibility. As a gentamycin system antibiotic to be used, gentamycin C1 (C21H43N 5O7), gentamycin C2, gentamycin C1a, etc. are included. As for a gentamycin system antibiotic, it is desirable to be contained in a culture medium in the amount more than 6.25microper 1ml of culture media g. Moreover, in order to avoid growth control of the drug-resistant strain at the time too of many, below 25microg is desirable. The optimum range of the amount used is 6.25-12.5microg per 1ml of culture media.

[0012] In this invention, the culture medium for cultivating Staphylococcus alternatively means the culture medium containing the component idiomatically used for the culture medium well-known to this contractor for the Staphylococcus culture. As such a component, casamino acids, heart extractives (heart infusion), various peptones, a yeast extract, a meat extract, the water soluble chlorophyll derivatives, grape sugar, L-tryptophan, L-cystine, a biotin, CaCl2, MgCl2, NaCl, a pyruvic acid (sodium), yolk liquid, an agar, mannite, coloring matter, etc. are mentioned. These components can be added to purified water and a culture medium can be manufactured.

[0013] Since it is desirable to use it combining an egg yolk reaction and a mannite reaction in order to distinguish multiple drug resistance Staphylococcus (for example, CNS) of MRSA and others also in Staphylococcus especially, it is desirable that yolk liquid, mannite, and coloring matter are included in a

culture medium. As for yolk liquid, it is desirable to contain 1 to 10% (weight / culture-medium volume). Moreover, as for 5-20g / 1l. of culture media, and coloring matter (for example, bromcresol purple, Phenol Red, neutral red, bromthymol blue, etc.), it is [mannite] desirable to contain 10-30mg / 1l. of culture media. By the egg yolk reaction, others are distinguished as a cluster to which the perimeter of a cluster is not changed as a cluster where MRSA has the muddiness ring of the perimeter of a cluster, and the pearly luster ring of a perimeter [cluster] culture-medium front face. Moreover, by the mannite reaction, it is distinguished as a cluster to which MRSA changes as yellowing of the culture-medium color of the perimeter of a cluster, and others do not change a culture-medium color.

[0014] As an example of the concrete medium composition for the Staphylococcus culture, what added additional components, such as yolk liquid, salt, mannite, grape sugar, amino acid, and a pyruvic acid, is mentioned, for example to basal media, such as a Muller-Hinton medium, a TORIPUCHIKESUSOI culture medium, a heart infusion culture medium, and a nutrient agar medium.

[0015] As an example with the desirable presentation of the culture medium for the Staphylococcus culture, the following presentations are mentioned, for example. To 1000ml of purified water, namely, the casamino acids 14.8-18.2g, The heart extractives 2.7-3.3g, water soluble chlorophyll derivatives 1.45-1.65g, Grape sugars 5-15g, L-tryptophans 0.045-0.055g, The L-cystines 0.045-0.055g, CaCl2 0.166-0.202g, MgCl 20.188-0.23g, NaCl 6.8-8.3g, pyruvic-acid sodium 0-15g, 1 - 10% (weight/volume) of yolk liquid, Agars 13.5-16.5g, Mannites 5-20g, and 10-30mg of coloring matter are added.

[0016] pH of the culture medium of this invention is desirable, and 7.0-7.8, and optimal pH are 7.4**0.2.

[0017] The culture medium of this invention can be cultivated with a conventional method. For example, 32-38 degrees C of culture are usually especially performed for the culture medium which carried out the smear of the fungus body preferably around 35 degrees C for about 24 hours for 18 to 48 hours.

[0018]

[Example] The following examples explain this invention in more detail. In addition, the following culture medium was used in the example.

Plate agar A oxacillin of this invention 0.1g, NaCl 75g, pyruvic-acid sodium 1g, agar 15g, mannite 1000ml of purified water was added to 10g and bromcresol purple 20mg. 4mg, ceftizoxime 12.5mg, casamino acids 15.5g, heart extractives 3.0g, water soluble chlorophyll derivatives 1.5g, grape sugar 2.0g, L-tryptophan 0.05g, L-cystine 0.05g, CaCl2 0.15g, MgCl2 After sterilizing this, yolk liquid is added to 5% (weight/volume) of rate, it passed 20ml each on the petri dish, and it was solidified.

Plate agar B oxacillin of this invention 2.0g, L-tryptophan 0.05g, L-cystine 0.05g, 2 0.15g of CaCl(s), 2 0.1g of MgCl(s), NaCl75g, pyruvic-acid sodium 1g, agar 15g, mannite 10g and bromcresol purple 1000ml of purified water be added to 20mg. 4mg, ceftizoxime 12.5mg, gentamycin 12.5mg, casamino acids 15.5g, heart extractives 3.0g, water soluble chlorophyll derivatives 1.5g, grape sugar After sterilizing this, yolk liquid is added to 5% (weight/volume) of rate, it passed 20ml each on the petri dish, and it was solidified.

Plate agar A of the example of a comparison (a component equal to the culture medium of this invention is contained except an antibiotic not being included)

Casamino acids 15.5g, heart extractives 3.0g, water soluble chlorophyll derivatives 1.5g, grape sugar 2.0g, L-tryptophan 0.05g, L-cystine 0.05g, CaCl2 0.15g, MgCl2 0.1g, NaCl 75g, pyruvic-acid sodium 1g, agar 15g, mannite 10g and bromcresol purple 1000ml of purified water was added to 20mg. After sterilizing this, yolk liquid is added to 5% (weight/volume) of rate, it passed 20ml each on the petri dish, and it was solidified.

Plate agar B (mannite salt yolk nutrient) meat extract of the example of a comparison 1.0g, peptone 10.0g, mannite 10.0g, NaCl 75.0g, Phenol Red 0.025g and agar 1000ml of purified water was added to 15.0g (it markets from Far East drug industry incorporated company as a Far East mannite salt agar medium). After sterilizing this, yolk liquid is added to 5% (weight/volume) of rate, it passed 20ml each on the petri dish, and it was solidified.

The smear of 20 shares of Staphylococcus aurei and 20 shares of methicillin sensitive Staphylococcus

aureuses (it is hereafter written as MSSA) which have been proved that they are examples 1-2 and the example 1 of a comparison - 2MRSA was carried out to each of the plate agar of this invention, the plate agar A of the example of a comparison, and the plate agar B of the example of a comparison, culture was performed at 35 degrees C for about 24 hours, and growth of Staphylococcus aureus was considered. A result is shown in Table 1.

[0019]

[Table 1]

表 1

	培地	MRSA	MSSA
実施例1	本発明の平板培地A	20株	0株
実施例2	本発明の平板培地B	20株	0株
比較例1	比較例の平板培地A	20株	20株
比較例2	比較例の平板培地B	20株	20株

[0020] Although it had set to the plate agar of the example of a comparison and all trial strain was also increasing the gap, in the plate agar of this invention, only MRSA increased alternatively.

an example 3 and the example 3 of a comparison - 4 multiple drug resistance CNS it is -- ** -- 20 shares of CNS which has become clear, and 20 shares of CNS of methicillin susceptibility -- the plate agar of this invention, the example plate agar A of a comparison, and the example plate agar B of a comparison -- respectively -- alike -- the smear -- carrying out -- 35 degrees C -- about 24-hour culture -- carrying out -- CNS Growth was considered. A result is shown in Table 2.

[0021]

[Table 2]

表 2

	培地	多剤耐性CNS	メチシリン感受性CNS
実施例3	本発明の平板培地A	20株	0株
比較例3	比較例の平板培地A	20株	20株
比較例4	比較例の平板培地B	20株	20株

[0022] Methicillin resistance CNS which shows multiple drug resistance in the plate agar of this invention although it has set to the plate agar of the example of a comparison and all trial strain is also increasing the gap It increased alternatively.

In order to grasp the MRSA possession situation between example 4 hospital pursuers, ****** of five hospital pursuers (A, B, C, and D), the pharynx, and the skin front face of a palm were wiped with the sterilization cotton swab, and what unfolded this cotton swab to a 1ml sterilization physiological saline well was used as a specimen. The smear of the 0.1ml of this specimen was carried out to homogeneity on the plate agar front face of this invention, culture was performed at 37 degrees C for about 24 hours,

and growth of a bacillus was considered. in addition, all stocks are [in / cluster / of staphylococci other than MRSA / a rabbit plasma coagulation test (coagulase test)] negative -- multiple drug resistance CNS it is -- things were judged. A result is shown in Table 3.

[0023]

[Table 3]

表 3

部位	対象者	菌数	
		MRSA	CNS
鼻前庭	A	1	0
	B	1	5
	C	0	4
	D	7	9
	E	0	0
咽頭	A	0	0
	B	1	0
	C	0	0
	D	3	4
	E	0	0
手のひら	A	0	0
	B	0	3
	C	0	0
	D	0	0
	E	0	1

[0024]

[Effect of the Invention] According to this invention, it can be made to be able to increase alternatively and Staphylococcus in which multiple drug resistance is shown can be detected. Therefore, the culture medium of this invention is very useful.

[Translation done.]